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Mycobacteria-specific cytokine responses as correlates of treatment response in active and latent tuberculosis

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Summary *Objectives:* A biomarker indicating successful tuberculosis (TB) therapy would assist in determining appropriate length of treatment. This study aimed to determine changes in mycobacteria-specific antigen-induced cytokine biomarkers in patients receiving therapy for latent or active TB, to identify biomarkers potentially correlating with treatment success.
Methods: A total of 33 adults with active TB and 36 with latent TB were followed longitudinally over therapy. Whole blood stimulation assays using mycobacteria-specific antigens (CFP-10, ESAT-6, PPD) were done on samples obtained at 0, 1, 3, 6 and 9 months. Cytokine responses (IFN-γ, IL-1ra, IL-2, IL-10, IL-13, IP-10, MIP-1β, and TNF-α) in supernatants were measured by Luminex xMAP immunoassay.
Results: In active TB cases, median IL-1ra (with CFP-10 and with PPD stimulation), IP-10 (CFP-10, ESAT-6), MIP-1β (ESAT-6, PPD), and TNF-α (ESAT-6) responses declined significantly over the course of therapy. In latent TB cases, median IL-1ra (CFP-10, ESAT-6, PPD), IL-2 (CFP-10, ESAT-6), and IP-10 (CFP-10, ESAT-6) responses declined significantly.

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Conclusions: Mycobacteria-specific cytokine responses change significantly over the course of therapy, and their kinetics in active TB differ from those observed in latent TB. In particular, mycobacteria-specific IL-1ra responses are potential correlates of successful therapy in both active and latent TB.

Introduction

The new World Health Organization (WHO) targets for reducing tuberculosis (TB) incidence and prevalence rely heavily on identifying cases of active TB and providing effective treatment.[1] There is also increasing emphasis on identifying and treating latent TB infection (LTBI) in high-risk individuals.[2] A key component of both these strategies is ensuring adequate treatment. At present there is no reliable biomarker of treatment success.[3–5]

In active TB, therapeutic response is usually judged clinically, in conjunction with laboratory measures such as conversion of sputum smears from positive to negative, correlating with reduction in mycobacterial load. Polymerase chain reaction (PCR) based methods, including the Xpert MTB/RIF assay that is being rolled out globally, cannot provide any useful information about treatment response, as these detect both viable and non-viable mycobacteria.[6] A biomarker that predicts treatment response and can guide decisions on length of treatment would be a significant advance in combating the ongoing TB pandemic. Several studies have shown differences in mycobacteria-specific cytokine responses between patients with active TB and individuals with LTBI at presentation.[7–10] In addition, data from a small number of studies indicate that monitoring cytokine responses, including TNF-α, IP-10, IL-2 and IL-10 responses, may have some predictive ability in patients with active TB.[11–14] However, most of these studies were small, had other significant limitations, and reported conflicting results.[3]

In LTBI, a biomarker of treatment success would also be of great benefit. At present, there is no test to confirm *Mycobacterium tuberculosis* (Mtb) eradication at completion of LTBI treatment. While interferon-γ (IFN-γ) release assays (IGRAs) are useful for establishing the diagnosis of LTBI, longitudinal studies have shown that IGRAs are not suitable for monitoring treatment response.[15,16] Only a minority of patients with an initially positive categorical IGRA result convert to a negative result after completing LTBI treatment.[15,16] Furthermore, quantitative changes in IFN-γ responses in IGRAs following treatment are also inconsistent. Finally, tuberculin skin tests (TSTs) are also not useful for monitoring treatment response, as most patients remain TST positive for several years after completion of LTBI treatment.[17]

An important potential application of a biomarker of TB treatment success is the ability to individualise duration of therapy. In some patients, treatment could potentially be shortened, whilst in patients at risk of treatment failure, therapy could be prolonged. Individualised treatment has the potential to both reduce associated costs substantially and decrease the risk of drug-related adverse events.[18]

Our study aimed to determine changes in Mtb antigen-induced cytokine biomarker responses in patients receiving treatment for LTBI or active TB to identify potential biomarkers correlating with treatment success.

Methods

Participants

Adult participants starting treatment for suspected LTBI or active TB were recruited following informed consent. The study was conducted at the Royal Melbourne Hospital (Victoria, Australia), a large tertiary referral centre, over a 3-year period (March 2012 to August 2014). These patients were part of a larger cohort recruited into our study of diagnostic biomarkers for TB.[19] Patients receiving immunosuppressive medication or with a primary or secondary immunodeficiency were excluded, as were patients who had received antituberculous therapy for more than one week.

We recorded data on demographic and clinical characteristics on a standardised case report form. Demographic details included country and date of birth, TB exposure history, BCG immunisation status and clinical symptoms. In addition, past medical history, current medications, physical examination and radiological results were recorded.

Tuberculin skin tests and interferon-gamma release assays

All participants had a TST (5 Tuberculin Units PPD; Tubersol, Sanofi Pasteur, Toronto, Canada) done by a trained healthcare professional, with the exception of participants who had microbiologically-confirmed active TB at recruitment. A positive TST result was defined as induration $\geq$10 mm at 48–72 h. In addition, blood samples were collected in sodium heparin tubes for whole blood stimulation assays and for QuantiFERON-TB Gold-in-Tube (QFT-GIT) assays. QFT-GIT assays were processed in a fully accredited diagnostic laboratory, following the manufacturer's instructions.

Categorisation of participants and follow-up

Based on TST, QFT-GIT and microbiological results, participants were classified into the following well-defined diagnostic groups: **Group A** – active TB: defined as microbiologically-proven TB based on culture and/or PCR results, **Group B** – LTBI: defined as asymptomatic patients with a positive TST, a positive IGRA result and a normal chest X-ray.

Participants who did not fulfil the criteria for these two distinct diagnostic categories were excluded from further analyses. Participants with LTBI were treated with isoniazid for 9 months. Participants with active TB were treated